

CLAIMS

What is claimed is:

1. An isolated and purified genetic marker associated with SCN/SDS resistance in soybeans, said marker mapping to linkage group G in the soybean genome, said marker having a sequence selected from among any of SEQ ID NOs:1, 3, and 5.
2. An isolated and purified genetic marker associated with SCN/SDS resistance in soybeans, said marker mapping to linkage group A2 in the soybean genome, said marker having a sequence selected from among any of SEQ ID NOs:7, 9, and 11.
3. A plant, or parts thereof, which evidences an SCN/SDS resistance response comprising a genome, homozygous with respect to genetic alleles which are native to a first parent and nonnative to a second parent of the plant, wherein said second parent evidences significantly less resistant response to SCN/SDS than said first parent and said plant comprises alleles from said first parent that evidences resistance to SCN/SDS in hybrid combination in at least one locus selected from: a locus mapping to linkage group G and mapped by one or more of the markers set forth as SEQ ID NOs:1, 3, and 5, a locus mapping to linkage group A2 and mapped by one or more of the markers set forth as SEQ ID NOs:7, 9, and 11; or combinations thereof, said resistance not significantly less than that of the first parent in the same hybrid combination, and yield characteristics which are not significantly different than those of the second parent in the same hybrid combination.

4. A plant, or parts thereof, as claimed in claim 3 comprising the progeny of a cross between first and second inbred lines, alleles conferring SCN/SDS resistance being present in the homozygous state in the genome of one or the other or both of said first and second inbred lines such that the genome of said first and second inbreds together donate to the hybrid a complement of alleles necessary to confer the SCN/SDS resistance.

5. An SCN/SDS resistant hybrid plant, or parts thereof, formed with the plant of claim 4.

6. A plant, or parts thereof, formed by selfing the SCN/SDS resistant hybrid of claim 5.

7. An isolated and purified biologically active SCN/SDS resistance polypeptide.

8. The isolated and purified biologically active SCN/SDS resistance polypeptide of claim 7, wherein the encoded polypeptide comprises a soybean SCN/SDS resistance polypeptide.

9. The isolated and purified biologically active SCN/SDS resistance polypeptide of claim 7, or functional portion thereof, wherein the polypeptide comprises:

- (a) a polypeptide encoded by a nucleic acid sequence of SEQ ID NO:13;
- (b) a polypeptide encoded by a nucleic acid molecule that is substantially identical to SEQ ID NO:13 ;
- (c) a polypeptide having the amino acid sequence of SEQ ID NO:14.

- (d) a polypeptide that is a biological equivalent of a peptide having the amino acid sequence of SEQ ID NO:14; or
- (e) a polypeptide that is immunologically cross-reactive with an antibody that shows specific binding with a polypeptide of SEQ ID NO:14.

5

10. The isolated and purified biologically active SCN/SDS resistance polypeptide of claim 7, modified to be in detectably labeled form.

11. An isolated and purified nucleic acid molecule encoding a biologically active SCN/SDS resistance polypeptide.

10

12. The nucleic acid molecule of claim 11, wherein the encoded polypeptide comprises a soybean SCN/SDS resistance polypeptide.

15

13. The nucleic acid molecule of claim 11, further comprising an isolated soybean *rhg1* and SDS resistance gene, said gene capable of conveying *Heterodera glycines*-infestation resistance, *Fusarium solani*-infection resistance, or both *Heterodera glycines*-infestation resistance and *Fusarium solani*-infection resistance to a non-resistant soybean germplasm, said gene located within a quantitative trait locus mapping to linkage group G and mapped by genetic markers of SEQ ID NOs:1-6, said gene located along said quantitative trait locus between said markers.

20

14. The nucleic acid molecule of claim 11, further defined as comprising:

- (a) the nucleotide sequence of any of SEQ ID NO:13 or
- (b) a nucleotide sequence that is substantially identical to any of SEQ ID NO:13.

15. The nucleic acid molecule of claim 13, further defined as comprising a 20 base pair nucleotide sequence that is identical to a contiguous 20 base pair nucleotide sequence of SEQ ID NO:13.

16. The nucleic acid molecule of claim 14, wherein the nucleic acid
5 sequence comprises a DNA sequence that hybridizes to a nucleic acid
sequence as set forth as SEQ ID NO:13 under wash stringency conditions
represented by a wash solution having about 200 mM salt concentration and a
wash temperature of at least about 45°C, and that encodes an SCN/SDS
resistance polypeptide.

10 17. The nucleic acid molecule of claim 11, further defined as a DNA
segment.

18. The nucleic acid molecule of claim 11, further defined as
positioned under the control of a promoter.

15 19. The nucleic acid molecule of claim 18, wherein said DNA segment
and promoter are operationally inserted into a recombinant vector.

20. A recombinant host cell comprising the nucleic acid molecule of
claim 11.

21. A transgenic plant having incorporated into its genome a nucleic
acid molecule of claim 11, the nucleic acid molecule being present in said
20 genome in a copy number effective to confer expression in the plant of an
SCN/SDS resistance polypeptide.

22. Plant seeds, parts, or progeny of a plant as claimed in claim 20.

23. The nucleic acid molecule of claim 11, further comprising an
isolated soybean *Rhg4* gene, said gene capable of conveying *Heterodera*

glycines-infestation resistance to a non-resistant soybean germplasm, said gene located within a quantitative trait locus mapping to linkage group A2 and mapped by the AFLP markers of SEQ ID NOs:7-12, said gene located along said quantitative trait locus between said markers.

- 5 24. The isolated gene of claim 23, further comprising: ?
- (a) the nucleotide sequence of any one of SEQ ID NOs:16-19; or
- (b) a nucleotide sequence substantially similar to any one of SEQ ID NOs:16-19.
25. A transgenic plant comprising the isolated soybean *Rhg4* gene of
- 10 claim 23.
26. Seeds, parts or progeny of a plant as claimed in claim 25.
27. An isolated SCN/SDS resistance gene promoter region, or functional portion thereof, comprising a 4.5 kb fragment of soybean genomic clone 21d9A2 8F8 between *EcoRI* restriction sites.
- 15 28. An isolated promoter region of claim 27, comprising:
- (a) the nucleotide sequence of SEQ ID NO:15; or
- (b) a nucleotide sequence substantially identical to SEQ ID NO:15.
29. The isolated promoter region of claim 28 comprising a 20 base pair nucleotide sequence identical to a contiguous 20 base pair nucleotide
- 20 portion of SEQ ID NO:15.
30. A chimeric gene comprising the isolated promoter region of claim 27 operably linked to a heterologous nucleotide sequence.
31. A vector comprising the chimeric gene of claim 30.
32. A host cell comprising the chimeric gene of claim 31.

33. The host cell of claim 32, wherein the cell is a bacterial cell or a plant cell.

34. A transgenic plant comprising a plant cell of claim 33.

35. An assay kit for detecting the presence, in biological samples, of a nucleic acid molecule encoding an SCN/SDS resistance polypeptide, the kit comprising a first container that contains a nucleic acid probe identical or complementary to a segment of at least ten contiguous nucleotide bases of the nucleic acid molecule of the odd-numbered SEQ ID NOs:1-13.

36. The kit of claim 35, further comprising a detectable moiety.

37. The kit of claim 35, wherein the biological sample further comprises chromosomes, and wherein the nucleic acid probe hybridizes to a chromosome.

38. A method for determining the presence or absence of SCN/SDS resistance in a soybean plant, or part thereof, comprising:

(a) detecting a molecular marker linked to a quantitative trait locus associated with SCN/SDS resistance, wherein the molecular marker comprises a sequence set forth as any one of SEQ ID NOs:1, 3, 5, 7, 9, and 11; and

(b) determining the presence of SCN/SDS resistance as detection of the molecular marker of step (a) and determining the absence of SCN/SDS resistance as failure to detect the molecular marker of step (a).

39. The method of claim 38, further comprising:

- (a) preparing genomic DNA from the soybean plant, or part thereof;
and
- (b) detecting a molecular marker linked to a quantitative trait locus
associated with SCN/SDS resistance, wherein the molecular
marker comprises a sequence set forth as any one of SEQ ID
NOs:1, 3, 5, 7, 9, and 11; and
- (c) determining the presence of SCN/SDS resistance as detection of
the molecular marker of step (b) and determining the absence of
SCN/SDS resistance as failure to detect the molecular marker of
step (b).

40. The method of claim 38, wherein the detecting comprises a
PCR-based assay.

41. A method of reliably and predictably introgressing SCN/SDS
resistance into non-resistant soybean germplasm, the method comprising:

- (a) identifying one or more nucleic acid markers for marker assisted
selection among soybean lines to be used in a soybean breeding
program, wherein the nucleic acid markers map to linkage groups
G or A2 and wherein the nucleic acid markers are selected from
among any of SEQ ID NOs:1, 3, 5, 7, 9, and 11; and
- (b) introgressing said resistance into said non-resistant soybean
germplasm by performing marker-assisted selection.

42. The method of claim 41, wherein the soybean germplasm is
derived from the "Forrest" line, or descendant thereof.

43. A plant, seed, or tissue culture produced by the method of claim 41 wherein the plant, seed, or tissue culture is resistant to SCN/SDS infection.

44. A method of positional cloning of a nucleic acid, the method comprising:

- 5 (a) identifying a first nucleic acid genetically linked to a SCN/SDS resistance locus, wherein the first nucleic acid maps between two markers selected from among any of SEQ ID NOs:1, 3, 5, 7, 9, and 11; and
- (b) cloning the first nucleic acid.

10 45. The method of claim 44, wherein the first nucleic acid comprises the *rhg1* and SDS locus.

46. The method of claim 44, wherein the first nucleic acid comprises the Rhg4 locus.

47. The method of claim 44, further comprising hybridizing a second
15 nucleic acid comprising the locus to a genomic library and selecting a clone that hybridizes to the second nucleic acid and comprises a second locus that confers SCN/SDS resistance in a plant.

48. The method of claim 44, further comprising hybridizing a second
20 nucleic acid comprising the locus to a genomic library and selecting a clone that hybridizes to the second nucleic acid, wherein the genomic library is selected from the group consisting of a BAC soybean genomic library, a YAC soybean genomic library, and a P1 bacteriophage soybean genomic library.

49. The method of claim 44, further comprising identifying overlapping clones.

50. The method of claim 44, wherein the first nucleic acid is amplified by PCR prior to cloning of the first nucleic acid.

51. The method of claim 44, wherein the first nucleic acid is proximal to the selected locus.

5 52. The method of claim 44, further comprising identifying a coding region encoded by the first nucleic acid.

53. The method of claim 44, wherein the SCN/SDS resistance locus corresponds to a nucleic acid selected from among any of SEQ ID NOs:13 and 16-19.

10 54. A method for producing an antibody that specifically recognizes a SCN/SDS resistance polypeptide, the method comprising:

- (a) recombinantly or synthetically producing a SCN/SDS resistance polypeptide, or portion thereof;
- (b) formulating the polypeptide of (a) whereby it is an effective immunogen;
- 15 (c) administering to an animal the formulation of (b) to generate an immune response in the animal comprising production of antibodies, wherein antibodies are present in the blood serum of the animal; and
- 20 (d) collecting the blood serum from the animal of (c) comprising antibodies that specifically recognize a SCN/SDS resistance polypeptide.

55. An antibody produced by the method of claim 54.

56. A method for detecting a level of a SCN/SDS resistance polypeptide, the method comprising

- 5
- (a) obtaining a biological sample having peptidic material;
 - (b) detecting a SCN/SDS resistance polypeptide in the biological sample of (a) by immunochemical reaction with the antibody of claim 55, whereby an amount of a SCN/SDS resistance polypeptide in a sample is determined.

57. A method for identifying a substance that modulates a SCN/SDS resistance polypeptide function, the method comprising:

- 10
- (a) isolating a SCN/SDS resistance polypeptide encoded by the nucleotide sequence of SEQ ID NO:13; a polypeptide encoded by a nucleic acid molecule that is substantially identical to SEQ ID NO:13; a polypeptide having the amino acid sequence of SEQ ID NO:14; a polypeptide that is a biological equivalent of the polypeptide of SEQ ID NO:14; or a polypeptide which is immunologically cross-reactive with an antibody that shows specific binding with a polypeptide of SEQ ID NO:14;
- 15
- (b) exposing the isolated SCN/SDS resistance polypeptide to one or more candidate substances;
- 20
- (c) assaying binding of a candidate substance to the isolated SCN/SDS resistance polypeptide; and
 - (d) selecting a substance that demonstrates selective binding to the isolated SCN/SDS resistance polypeptide.

58. A method of detecting a nucleic acid molecule that encodes an SCN/SDS resistance polypeptide in a biological sample containing nucleic acid material, the method comprising:

- 5
- (a) hybridizing the nucleic acid molecule of claim 13 under stringent hybridization conditions to the nucleic acid material of the biological sample, thereby forming a hybridization duplex; and
 - (b) detecting the hybridization duplex, whereby a nucleic acid molecule encoding a SCN/SDS resistance polypeptide is detected in the biological sample.

10 59. The method of claim 58, wherein the nucleic acid molecule that encodes an SCN/SDS resistance polypeptide further comprises a chromosome.

60. A method for identifying soybean sudden death syndrome (SDS) resistance or soybean cyst nematode (SCN) resistance in a plant using a SDS resistance gene, a SCN resistance gene, or DNA segments having homology to
15 a SDS resistance gene or to an SCN resistance gene, the method comprising:

- (a) probing nucleic acids obtained from the plant with a probe derived from said SDS resistance gene or from said SCN resistance gene or from said DNA segment having homology to said SDS resistance gene or to said SCN resistance gene; and
- 20 (b) observing hybridization of said probe to said nucleic acids, the presence of said hybridization indicating SDS or SCN resistance in said plant.

61. The method of claim 60, wherein the probe comprises an isolated and purified nucleic acid molecule encoding a biologically active SCN/SDS resistance polypeptide.

62. The method of claim 60, wherein the probe comprises a nucleotide
5 sequence as set forth in of any of SEQ ID NOs:13 and 16-19, or any complementary strand thereof, or any combination thereof.

63. A method for identifying a candidate compound as a modulator of SCN/SDS resistance activity, the method comprising:

- 10 (a) exposing a cell sample with a candidate compound to be tested, the cell sample containing at least one cell containing a DNA construct comprising a modulatable transcriptional regulatory sequence of an SCN/SDS resistance-encoding nucleic acid and a reporter gene which is capable of producing a detectable signal;
- 15 (b) evaluating an amount of signal produced in relation to a control sample; and
- (c) identifying a candidate compound as a modulator of SCN/SDS resistance activity based on the amount of signal produced in relation to a control sample.

64. The method of 63, wherein the reporter gene comprises a nucleic
20 acid molecule encoding an SCN/SDS resistance polypeptide.

65. The method of claim 63, wherein the modulatable transcriptional regulatory sequence comprises SEQ ID NO:15.

66. A method of modulating SCN/SDS resistance in a plant, the method comprising administering to the plant an effective amount of a

substance that modulates expression of an SCN/SDS resistance activity-encoding nucleic acid molecule in the plant to thereby modulate SCN/SDS resistance in the plant.

67. The method of claim 66, wherein the substance that modulates
5 expression of an SCN/SDS resistance activity-encoding nucleic acid molecule comprises a ligand for a regulatory protein that binds a SCN/SDS resistance gene promoter.

68. The method of claim 67, wherein the SCN/SDS resistance gene
10 promoter comprises the nucleotide sequence of SEQ ID NO:15, or functional portion thereof.

69. A method for modulating SCN/SDS resistance in a plant, the method comprising administering to the plant an effective amount of a substance that modulates SCN/SDS resistance polypeptide activity to thereby modulate SCN/SDS resistance in the plant.

15 70. The method of claim 69, wherein the plant is a soybean plant.

71. A method for providing a resistance trait to a plant, the method comprising introducing to said plant a construct comprising a nucleic acid sequence encoding an SCN/SDS resistance gene product operatively linked to a promoter, wherein production of the SCN/SDS resistance gene product in the
20 plant provides SCN or SDS resistance trait to the plant.

72. The method of claim 71, wherein the construct further comprises a vector selected from the group consisting of a plasmid vector or a viral vector.

73. The method of claim 71, wherein the SCN/SDS resistance gene product comprises a protein having an amino acid sequence of SEQ ID NO:14.

74. The method of claim 71, wherein the nucleic acid sequence is selected from the group consisting of:

- (a) a nucleotide sequence set forth as SEQ ID NO:13;
- (b) a nucleotide sequence substantially similar to SEQ ID NO:13.

5 75. The method of claim 71, wherein the resistance characteristic is nematode resistance, fungal resistance or combinations thereof.

76. The method of claim 75, wherein the nematode resistance is *H. glycines* resistance.

10 77. The method of claim 76, wherein the *H. glycines* resistance is race 3 *H. glycines* resistance.

78. The method of claim 71, wherein the construct further comprises another nucleic acid molecule encoding a polypeptide that provides an additional desired characteristic to the plant.

15 79. The method of either of claims 71 or 78, wherein the method further comprises monitoring an insertion point for the construct in the plant genome; and providing for insertion of the construct into the plant genome at a location not associated with the resistance characteristic, the desired characteristic, or both the resistance or the desired characteristic.

80. The method of claim 71, wherein the plant is a soybean plant.